



Genetic variants in exon 1 region of *FABP3* gene in relation to milk production traits in Sahiwal and Karan Fries cattle

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ABSTRACT. The present study pertained to records on milk production and milk constituents of 100 Sahiwal cattle and 115 Karan Fries cattle, the data collected over a period of 2004 to 2016 from Animal Genetics and Breeding division from ICAR-National Dairy Research Institute, Karnal, Haryana. In Sahiwal SNP at position G649C was found to be highly associated with FLTMY and FL305DFY and however the association was non-significant for FL305DMY, FL305DSNFY and FL305DPY. GC genotype was superior with respect to FL305DPY and FL305DFY traits, whereas, homozygous (GG) genotype was superior for rest of other traits. In Karan Fries SNP at position G649C was found to be highly associated with FL305DFY and however, the association was non-significant for FL305DMY, FLTMY, FL305DSNFY and FL305DPY. Heterozygote GC genotype was superior for all traits; however the superiority is significantly higher for FL305DFY traits in present study.

Keywords: Sahiwal and Karan Fries cattle; *FABP3* gene; Milk constituent's traits

INTRODUCTION

India is a rich reservoir of genetic diversity in cattle with 41 recognized cattle breeds. The total number of cattle population was 190.90 million (37.28% of total livestock population) out of which indigenous cattle population comprised of 151.17 million (Livestock Census, 2012). As far as milk production is concerned Sahiwal is the best dairy breed of the Indian subcontinent. The Karan Fries breed has been evolved from crossbreeding between Tharparkar and Holstein Friesian at the ICAR-National Dairy Research Institute, Karnal, Haryana. The FABPs (Fatty Acid Binding Proteins) are 14-15 kDa protein that reversibly binds non-esterified saturated and unsaturated long-chain fatty acids, eicosanoids and other lipids with the purpose of transporting or storing them inside a cell (Shimano H et al., 1996). There are nine members of the FABPs protein family that are expressed differentially in multiple tissues. The most abundant isoforms of FABPs expressed in the lactating bovine mammary gland are *FABP3*, *FABP4* and *FABP5* (Jensen RG, 2002). The *FABP3* protein, also known as 'heart FABP', is expressed in tissues such as heart, skeletal muscle, mammary gland, brown adipose tissue and other tissues (Shimano H et al., 1996). The main function of *FABP3* is to channel fatty acids inside a cell towards mitochondrial β -oxidation in heart and skeletal muscles. The discovery of *FABP3* protein in mammary gland was related to the identification of a Mammary Derived Growth Inhibitor (MDGI) that turned out to be a mixture of *FABP3* and *FABP4* proteins (Yang T et al., 2002); (German JB and Dillard CJ, 2006). The *FABP3* protein is highly expressed in the mammary gland during cell differentiation and formation of ductal structures at the onset of lactation (Binas B et al., 1992), but the requirement of *FABP3* for mammary tissue development and function is not well established (Schaefer EJ, 2002; Stoop WM et al., 2008). The *FABP3* highly expressed in bovine mammary gland during lactation and being the second most abundant transcript among all measured. The polymorphisms in *FABP3* genes affect the selectivity of fatty acid uptake from the blood and fatty acid transport inside the mammary epithelial cells, resulting in differences in milk fatty acid composition (Jensen RG, 2002; Soyeyrt H et al., 2006).

MATERIAL AND METHODS

Experimental animals and genomic DNA isolation

The data for present study pertained to various milk production and milk constituents traits were collected from history sheets and milk constituents registers, data on milk production and milk constituents records of 100 Sahiwal and 115 Karan Fries cattle with spread over a period of 13 years from 2004 to 2016 were collected from Animal Genetics and Breeding division of ICAR-National Dairy Research Institute, Karnal, Haryana. Blood samples were collected from the selected population. About 10 ml of venous blood was collected aseptically from the jugular vein of the animals in a 15ml polypropylene centrifuge tube under sterile condition using 0.5 ml of EDTA as an anticoagulant. The tube was shaken gently to facilitate thorough mixing of blood with the anticoagulant. The tubes containing blood samples were transported to the laboratory as soon as possible in an icebox containing ice packs and were stored in the refrigerator at -20°C temperature until the isolation of DNA was done. Phenol extraction method as described by Sambrook and Russell (Sambrook J and Russell DW, 2001) was used for isolation of genomic DNA. Horizontal submarine agarose gel electrophoresis was used to check the quality of genomic DNA. The purity of genomic DNA was checked by spectrophotometer. The 6 μl of genomic DNA of each sample was dissolved in 294 μl of triple distilled water and spectrophotometer readings at OD260 and OD280 was recorded against 300 μl double distilled water as a blank. Genomic DNA samples showing the OD ratio in the range of 1.7 to 1.9 was used further in the study.

Amplification of targeted region of *AAT* genes

Only good quality genomic DNA was used for amplification of Exon 1 of *FABP3* gene (423bp) by polymerase chain reaction under optimized conditions.

F: 5' -CTGACGTAGGCAAACCTGGGT -3'

R: 5' - CATGCAGGATCTAGCCAGGG -3'

Primers (F) and (R) was used to amplify Exon 1 region of *FABP3* gene, which were designed to amplify genes using Primer 3 software (<http://www.primer3.ut.ee>) (Untergasser A et al., 2012) and gene sequence available at NCBI database (<http://www.ncbi.nlm.nih.gov>). The primers designed were checked for specificity by BLAST (version 1.2.0). Primers were designed and synthesized from Sigma Aldrich Chemicals Pvt.Ltd (USA), the best amplification of the desired fragment was taken for further analysis. The standard programme is given as under (Table 1):

Table 1: The standard programme for PCR Reaction mix (25 µl).

Polymerase chain reaction amplification of exon 1 region of *FABP3* genes

Various annealing temperatures were tried for PCR amplification of *FABP3* genes. Total volume of 25 µl for each sample was used to set up the PCR reactions. The set of primer was used to amplify target regions of *FABP3* genes in Sahiwal and Karan Fries cattle. The best results were obtained when amplification was performed in PCR thermal cycler (Eppendorf Germany) programmed for 32 cycles with an initial denaturation at 94°C for 05 minutes, denaturation at 94°C for 30 second, annealing at 62°C for 30 second and extension at 72°C for 30 second with a final extension at 72°C for 10 minutes.

Agarose gel electrophoresis of polymerase chain reaction product

Components	Concentration	Volume (µl)
1. 10x Buffer	10 X	2.5
2. 1.5 mM MgCl ₂	15 mM	1.5
3. d NTP's	200 µM	0.5
4. F-primer	30 pm	3.0
5. R-primer	30 pm	3.0
6. Taq (5 units/µl)	1 unit	0.2
7. Template	100 ng	1.0
8. Distilled water	--	14.3

The amplified product was checked for quality and quantity by agarose gel electrophoresis as described by Sambrook and Russell, 2001. Total 5 µl of amplified PCR product of each sample was mixed with 1 µl of 6x gel loading dye buffer from each tube. The samples were loaded on 2% agarose gel containing ethidium bromide (1% solution @ 5 µl /100 ml) along with 100 bp DNA ladder (O'GeneRuler™-Fermentas) at a constant voltage of 70V for 45 minutes in 0.5x TBE buffer. The amplified PCR product on agarose gel was visualized as a single compact band of different primers size under UV trans-illuminator and documented by photograph through gel documentation system (Bio-Rad, USA) (Plates 1 and 2).

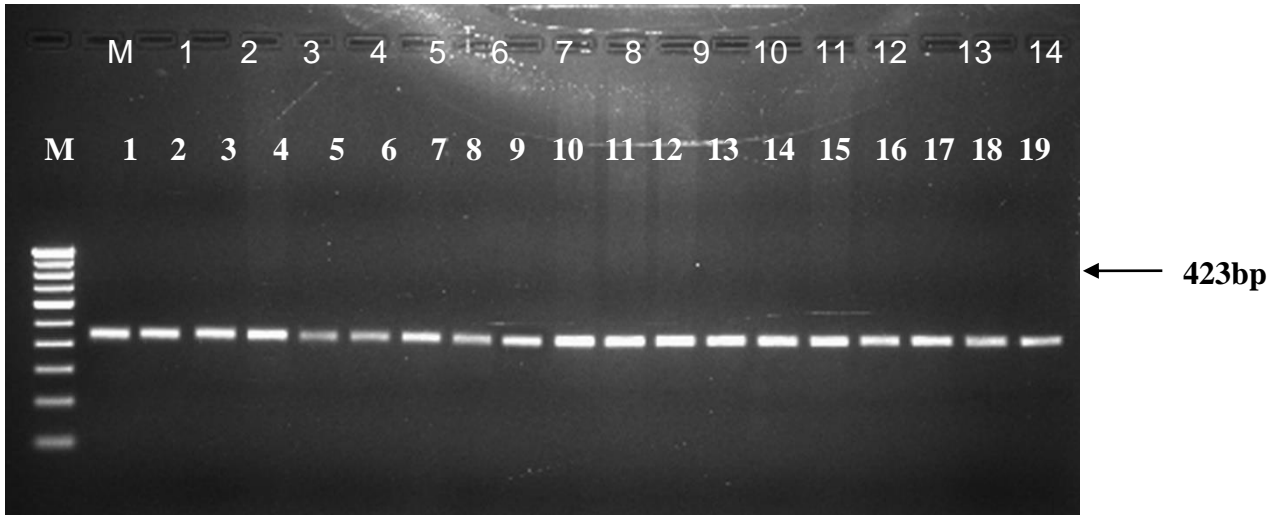


Plate 1: Amplified PCR product of 423bp of *FABP3* gene electrophoresed on 2% agarose in Sahiwal cattle.

M: 100 bp DNA ladder.

Lanes: 1-19 are amplified PCR product

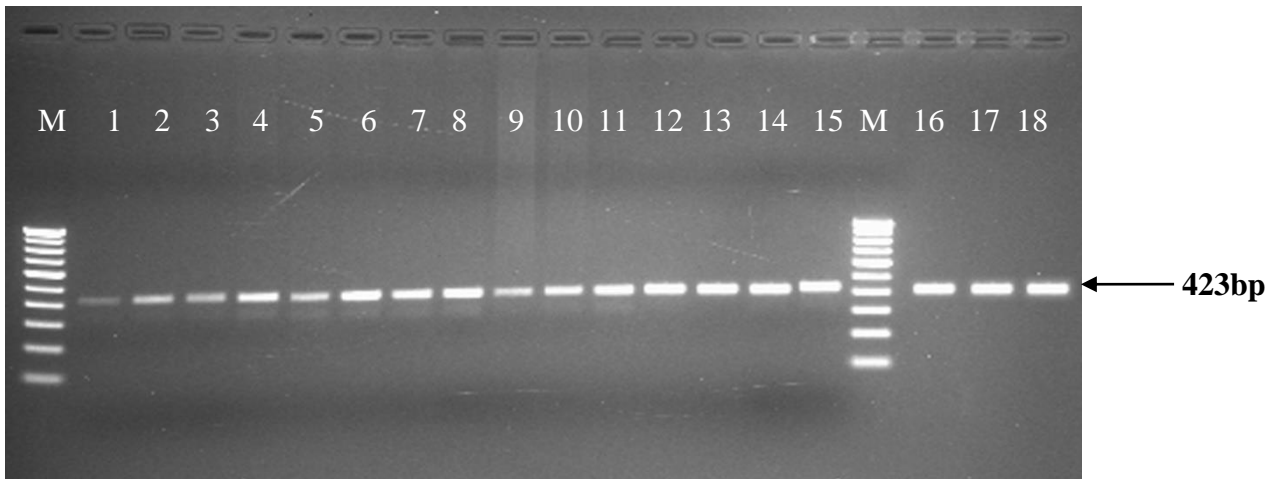


Plate 2: Amplified PCR product of 423 bp of *FABP3* Gene electrophoresed on 2% agarose in Karan Fries cattle.

M: 100 bp DNA ladder

Lanes: 1-18 are amplified PCR product

Sequenced data analysis for SNPs detection

For custom sequencing ten amplicons of different sizes were sent to 1st BASE Sequencing INT by using forward and reverse primers and the final sequences of each contig for Sahiwal and Karan Fries cattle were deduced from the raw sequences by using Bioedit software. Amplified PCR products were sequenced and after Clustal W analysis (www.ebi.ac.uk/tools/msa/clustalw2) (Larkin MA, et al., 2007). ClustalW software was used for determining the SNPs in which complete coding sequence of animal were compared and aligned with the edited sequences of other Sahiwal and Karan Fries cattle (Figures 1 and 2).

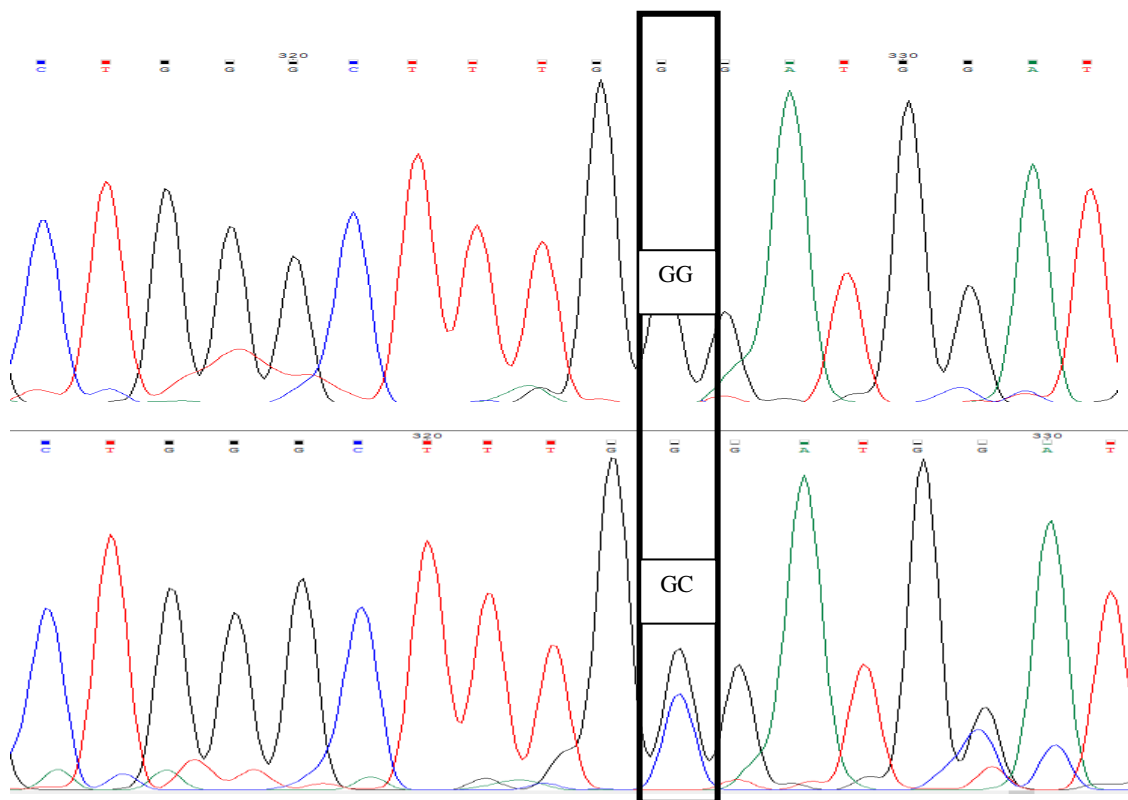


Figure 1: Chromatogram showing change at G649C of *FABP3* Gene (423 bp) in Sahiwal cattle.

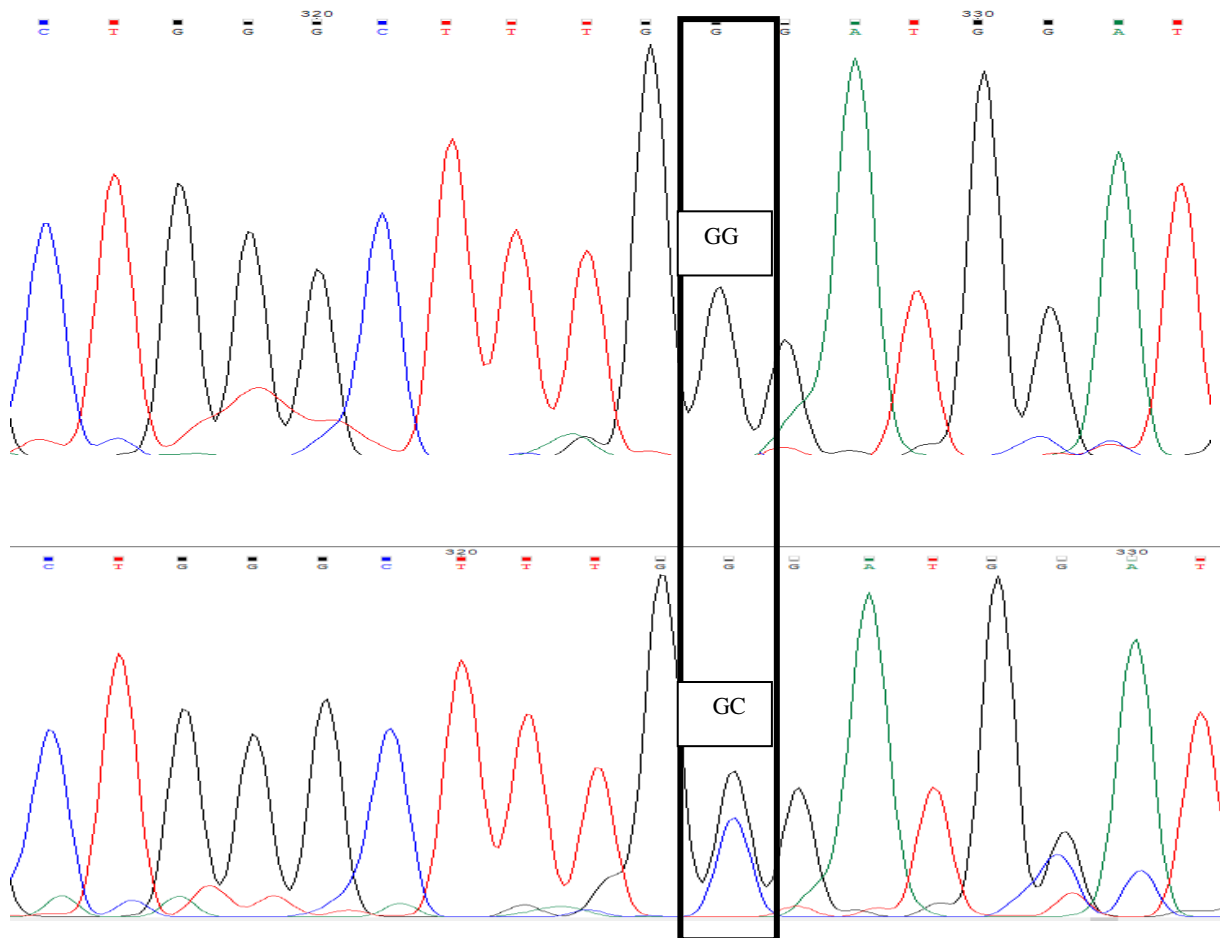


Figure 2: Chromatogram showing change at G649C of *FABP3* gene (423 bp) in Karan Fries cattle.

Statistical analysis of PCR-RFLP data

The analysis was carried out with appropriate Statistical method using software's in the computer centre of the institute under the following headings:

Restricted Maximum Likelihood Method (REML)

Estimation of breeding value: The single trait animal model was considered for estimation of breeding value using WOMBAT software (Meyer K 2010). The following animal model was considered:

$$Y_{ijk} = X b_i + Z u_j + e_{ijk}$$

Where,

- Y_{ijk} = k^{th} observation of j^{th} random effect of i^{th} fixed effect
 b_i = Vector of observation of fixed effect
 X = Incidence matrix of fixed effect
 u_j = Vector of additive genetic effect (Animal effect)
 Z = Incidence matrix of random effect
 e_{ijk} = Vector of residual errors

Association estimation: Based on the adjusted records, pertaining to milk yield and its constituents on Sahiwal and Karan Fries cattle maintained at ICAR-NDRI, Karnal, regression analysis was carried out to identify SNPs contributing significantly to the variation in milk and its constituents.:

$$Y_{ijk} = a + b_1 \text{SNP}_1 + b_2 \text{SNP}_2 + \dots + b_n \text{SNP}_n + e_{ijk}$$

where,

- Y_{ijk} = Adjusted observation on k^{th} animal of i^{th} , j^{th} ... n^{th} SNPs
 a = Intercept
 $b_1 \dots n$ = Partial regression coefficient for SNPs considered
 $\text{SNP}_{1,j \dots n}$ = Effect of SNPs taken as independent variable
 e_{ijk} = Random error NID (0, σ^2_e)

Effect of genotypes on breeding value: The relative contribution of Genotypes to breeding value of the animal for milk yield and milk constituents was assessed using the following model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where,

- Y_{ij} = Breeding value of j^{th} animal of i^{th} genotype
 μ = Overall mean
 G_i = Effect of i^{th} genotypes (SNPs/haplotypes)
 e_{ij} = Residual error NID (0, σ^2_e)

Result and Discussion

Genetic variant (SNP) at G649C location of exon 1 of *FABP3* Gene (423 bp) in Sahiwal. In Sahiwal SNP at position G649C was found to be highly associated with first lactation total milk yield (FLTMY) and First Lactation 305 day's fat yield (FL305DFY) and however the association was non-significant for First Lactation 305-days milk yield (FL305DMY), First Lactation 305 days SNF yield (FL305DSNFY) and First Lactation 305 days protein yield (FL305DPY). The mean \pm SE of GG genotype for FL305DMY, FLTMY, FL305DFY, FL305DSNFY, FL305DPY were found to be 1827.7 ± 12.8 , 2039.2 ± 12.7 , 97.63 ± 0.42 , 155.06 ± 0.14 and 43.85 ± 0.12 respectively in the present study and for Heterozygous (GC) genotype were 1802.24 ± 8.37 ,

1998.61 ± 8.31, 101.50 ± 0.28, 154.81 ± 0.09 and 43.92 ± 0.08 respectively (Tables 2 and 3). GC genotype was superior with respect to FL305DPY and FL305DFY traits, whereas, homozygous (GG) genotype was superior for rest of other traits. The result is also in agreement with Kulig et al., 2010 and reported AA genotypes were characterized by a significantly 0.08% higher protein content in milk than AG and 0.07% higher than GG genotypes. Milk fat content in AA cows was significantly higher compared with AG and GG cows by 0.31 and 0.46 % respectively. AG cows were characterized by a significantly 0.15% higher fat content than GG cows. Out of total variability in fat 4.2% was explained by *FABP3* polymorphism, protein 2.2% by *FABP3* genotype. *FABP3* significantly affects fat and protein content in Jersey cow with A as a desirable allele for improving these traits. *FABP3* gene can be considered marker for milk production traits. Nafikovet et al., 2013 reported overall haplotype effect of *FABP3* was associated significantly with the concentrations of only pentadecylic (15:0) acid and elongation index.

Table 2: ANOVA for SNP G649C of *FABP3* gene in Sahiwal cattle.

Source	df	SS	MSS	F-value
FL305D MY				
SNP G649C	1	13652	13652	2.79
Error	98	480149	4899	
Total	99	493801		
FLTMY				
SNP G649C	1	34554	34554	7.15**
Error	98	473800	4835	
Total	99	508355		
FL305DFY				
SNP G649C	1	314.82	314.82	56.89**
Error	98	542.27	5.53	
Total	99	857.09		
FL305DSNFY				
SNP G649C	1	1.33	1.33	2.28
Error	98	57.55	0.58	
Total	99	58.89		
FL305DPY				
SNP G649C	1	0.11	0.11	0.23
Error	98	46.84	0.47	
Total	99	46.95		

**Highly significant (p<0.01)

Table 3: Least square mean and standard error for milk production traits.

SNP G649C	N	Mean (Kg) ± SE
FL305DMY		
GC	70	1802.24 ± 8.37
GG	30	1827.7 ± 12.8
FLTMY		
GC	70	1998.61 ± 8.31 ^a
GG	30	2039.2 ± 12.70 ^b
FL305DFY		
GC	70	101.50 ± 2.81 ^a
GG	30	97.63 ± 4.29 ^b
FL305DSNFY		

GC	70	154.81 ± 9.20
GG	30	155.06 ± 14.0
FL305DPY		
GC	70	43.92 ± 8.26
GG	30	43.85 ± 12.60

Regression equation: The significance of association of SNP with different performance traits were estimated by constructing the regression equation and the best fit equation for each of them is given below:

$$\text{FL305DMY} = 1814.99 - 12.75 \text{ SNP_GC} + 12.75 \text{ SNP_GG} \text{ (R}^2 = 2.76)$$

$$\text{FLTMY} = 2018.89 - 20.28 \text{ SNP_GC} + 20.28 \text{ SNP_GG} \text{ (R}^2 = 6.80)$$

$$\text{FL305DFY} = 99.56 + 1.93 \text{ SNP_GC} - 1.93 \text{ SNP_GG} \text{ (R}^2 = 36.73)$$

$$\text{FL305DSNFY} = 154.94 - 0.12 \text{ SNP_GC} + 0.12 \text{ SNP_GG} \text{ (R}^2 = 2.27)$$

$$\text{FL305DPY} = 43.89 + 0.03 \text{ SNP_GC} - 0.03 \text{ SNP_GG} \text{ (R}^2 = 0.24)$$

Genetic variant at SNP G649C location in exon 1 of *FABP3* gene in Karan Fries cattle

SNP at position G649C was found to be highly associated with FL305DFY and however the association was non-significant for FL305DMY, FLTMY, FL305DSNFY and FL305DPY. The mean ± SE of homozygous (GG) genotype for FL305DMY, FLTMY, FL305DFY, FL305DSNFY, FL305DPY were found to be 3513.50 ± 10.7, 4533.30 ± 10.7, 97.75 ± 3.81, 278.04 ± 0.10 and 113.25 ± 0.10 respectively in the present study and for Heterozygous GC genotype were 3429.23 ± 8.59, 4549.44 ± 8.59, 159.75 ± 3.05, 278.19 ± 0.08, 113.48 ± 0.08 respectively (Tables 4 and 5). Heterozygote GC genotype was superior for all traits; however the superiority is significantly higher for FL305DFY traits in present study. The result is also in agreement with Kulig et al., 2010 and reported AA genotypes were characterized by a significantly 0.08% higher protein content in milk than AG and 0.07% higher than GG genotypes. Milk fat content in AA cows was significantly higher compared with AG and GG cows by 0.31 and 0.46 % respectively. AG cows were characterized by a significantly 0.15% higher fat content than GG cows. Out of total variability in fat 4.2% was explained by *FABP3* polymorphism, protein 2.2% by *FABP3* genotype. *FABP3* significantly affects fat and protein content in Jersey cow with A as a desirable allele for improving these traits. *FABP3* gene can be considered marker for milk production traits. Nafikovet et al. 2013 reported overall haplotype effect of *FABP3* was associated significantly with the concentrations of only pentadecylic (15:0) acid and elongation index.

Table 4: ANOVA for SNP G649C of *FABP3* Gene in Karan Fries

Source	df	SS	MSS	F-value
FL305D MY				
G649C	1	7144	7144	1.38
Error	113	583867	5167	
Total	114	591011		
FLTMY				
G649C	1	7144	7144	1.38
Error	113	583867	5167	
Total	114	591011		
FL305DFY				
G649C	1	105293.60	105293.60	161.39**
Error	113	7372.20	652.40	
Total	114	179015.81		

FL305DSNFY				
G649C	1	0.64	0.64	1.29
Error	113	56.31	0.49	
Total	114	56.95		
FL305DPY				
G649C	1	1.34	1.34	2.50
Error	113	60.62	0.53	
Total	114	61.97		

**Highly significant ($p < 0.01$)

CONCLUSION

In Sahiwal SNP at position G649C was found to be highly associated with FLTMY and FL305DFY. GC genotype was superior with respect to FL305DPY and FL305DFY traits, whereas, homozygous (GG) genotype was superior for rest of other traits. In Karan Fries cattle SNP at position G649C was found to be highly associated with FL305DFY. Heterozygote GC genotype was superior for all traits; however the superiority is significantly higher for FL305DFY traits in present study.

Table 5: Least square mean and standard error for milk production traits.

G649C	N	Mean (Kg) \pm SE
FL305DMY		
GC	70	3529.68 \pm 8.59
GG	45	3513.50 \pm 10.70
FLTMY		
GC	70	4549.44 \pm 8.59
GG	45	4533.3 \pm 10.70
FL305DFY		
GC	70	159.75 \pm 3.05 ^a
GG	45	97.75 \pm 3.81 ^b
FL305DSNFY		
GC	70	278.19 \pm 8.40
GG	45	278.04 \pm 10.50
FL305DPY		
GC	70	113.48 \pm 8.80
GG	45	113.25 \pm 10.90

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